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## Measures of cardiovascular autonomic nervous function: agreement, reproducibility, and reference values in middle age and elderly subjects

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### Abstract

**Aims/hypothesis.** Currently, three categories of cardiovascular autonomic nervous function measures are used: classic Ewing-test measures, measures of heart-rate variability (HRV), and measures of baroreflex sensitivity (BRS). Little is known about the agreement between these measures, and reference and reproducibility values for these measures have not been reported within the same group.

**Methods.** As part of the Hoorn Study, 631 subjects aged 50 to 75 participated in a study of autonomic nervous function. Cardiac cycle duration (RR interval) and continuous finger arterial pressure were measured under three conditions: during spontaneous breathing, during six deep breaths over 1 min, and during an active change in position from lying to standing. From these readings, ten measures of autonomic function were assessed (mean heart rate, three Ewing test measures, five HRV measures and one BRS measure).

**Results.** Regression analysis in a healthy subgroup ( $n=191$ ) showed sex differences for two of the ten

measures and seven measures decreased with age. Therefore, appropriate age-specific and sex-specific reference values were calculated. Reproducibility ( $n=39$ ) of most measures was moderate, with a reliability coefficient of around 50%. Agreement between the measures of autonomic nervous function varied greatly, between 0% and 87%. The HRV-power ratio measure and the blood pressure changes in the lying-to-standing test showed the lowest agreement with all other measures.

**Conclusion/Interpretation.** This study provides age-specific and sex-specific reference values for a wide range of different autonomic function measures in an elderly population. Agreement among the different measures varied widely and reproducibility was only moderate. [Diabetologia (2003) 46:330–338]

**Keywords** Aging, agreement, baroreflex, diabetes mellitus, heart-rate variability, nervous system, autonomic, reference values, reproducibility.

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**Abbreviations:** BRS, Baroreflex sensitivity (ms/mmHg); DM, Diabetes mellitus; EI difference, Expiration-inspiration difference in RR intervals during breathing at 6/min (ms); HRV, Heart-rate variability; HF power, High frequency power in the RR-interval spectrum between 0.12–0.40 Hz ( $\text{ms}^2$ ); KDM, Known diabetes mellitus; LF power, Low-frequency power in

the RR-interval spectrum between 0.04–0.12 Hz ( $\text{ms}^2$ ); LF/(LF+HF), Ratio of low frequency power to the sum of low and high frequency power in the RR-interval spectrum; Mean NN, Mean of all sinus rhythm (normal-to-normal) RR intervals (ms); NDM, Newly-diagnosed diabetes mellitus; NGT, Normal glucose tolerance; RRmax, Maximal change in RR interval after standing up (ms); RRmax/min, Maximal RR interval between 15 s and 30 s after standing up divided by the minimal RR interval within 15 s after standing up; SBP difference, Systolic blood pressure 1.5–2 minutes after standing up minus systolic blood pressure in supine position (mmHg); SDNN, Standard deviation of all sinus rhythm (normal-to-normal) RR intervals (ms).

Autonomic changes in the heart and cardiovascular system in diabetes mellitus occur as part of the wider spectrum of autonomic neuropathy, which affects most organs of the body, e.g. the gastrointestinal and the genitourinary tracts, the sudomotor system involved in sweat production, the eyes and the endocrine organs. It is in the cardiovascular system that involvement of the autonomic nervous system is most noticeable and most easily assessed. Abnormalities can be detected in an early, asymptomatic phase and in a non-invasive way. Therefore, cardiovascular autonomic function tests could play an important role in the early detection of (diabetic) autonomic neuropathy. To some extent consensus was obtained in the past on a number of tests to assess cardiovascular autonomic function [1, 2]: the so-called Ewing battery [3]. In addition, heart-rate variability (HRV) and, more recently, the sensitivity of spontaneous baroreflex control of the heart rate (baroreflex sensitivity, BRS), have been used to assess autonomic dysfunction as well. It has been proposed that these measures might be even more sensitive than the Ewing battery [4, 5, 6, 7].

Among the standard cardiovascular autonomic function tests, i.e. the Ewing battery, are the deep breathing test, the lying-to-standing test, the Valsalva manoeuvre and the sustained handgrip test [3]. These Ewing test measures, and also HRV and BRS measures, are based on the autonomically mediated response of the heart rate to changes in blood pressure. Autonomic function has been reported to decrease in relation to age [8, 9, 10, 11, 12, 13] and to differ between men and women [9, 14, 15]. Furthermore, diabetic patients have lower values on the Ewing battery [3, 16, 17, 18, 19], of the spectral analysis of HRV [4, 16, 20, 21], and have a low BRS [5, 22] in comparison with normal glucose tolerant control subjects. Besides their application in the assessment of cardiovascular autonomic function in diabetic patients, HRV and BRS are used in cardiology for risk stratification after myocardial infarction. Low HRV is associated with mortality risk in post-myocardial infarction patients [23, 24] and also in the general population [25, 26]. Also, autonomic neuropathy has been proposed to play a role in the aetiology of cardiovascular disease [27, 28, 29, 30, 31] and nephropathy [32, 33] in diabetes. So far, the agreement between the autonomic functions tests, their reproducibility and reference values have not been reported in a single study. This information will be valuable for clinical practice [34].

## Subjects and methods

*Study population: reference values and agreement.* The Hoorn Study is a prospective study of glucose tolerance and cardiovascular risk factors in a 50 to 75-year-old general Caucasian population [35, 36, 37, 38]. In short, an age-, sex- and glucose-tolerance-stratified sample of 708 subjects out of a cohort of 2484 was invited for a second 75-gram oral glucose tolerance

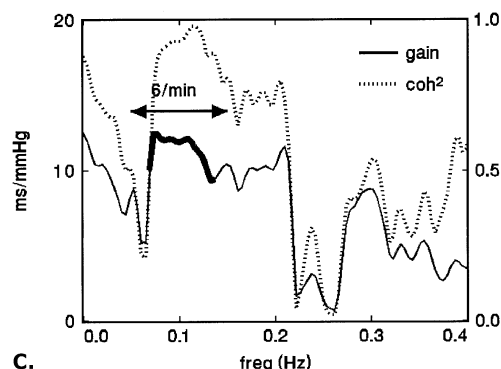
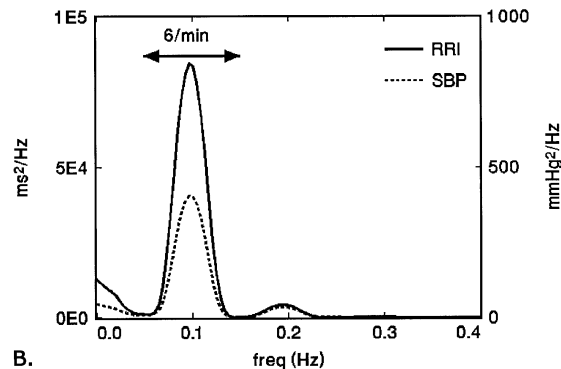
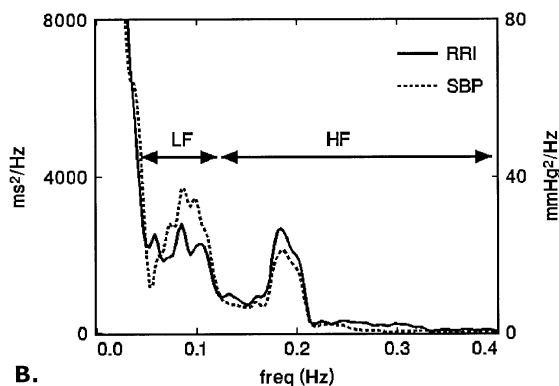
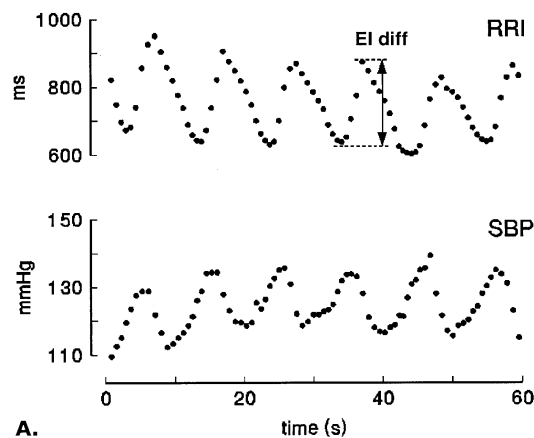
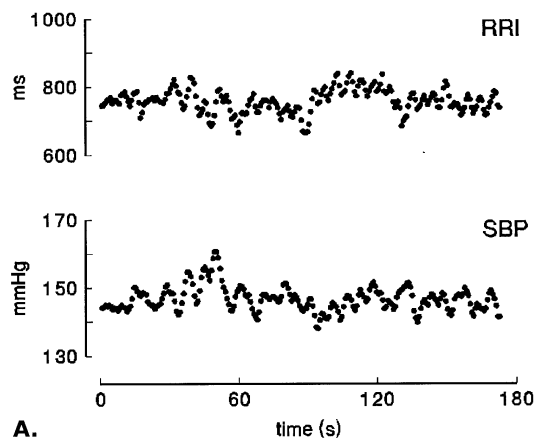
test within 3 to 5 weeks and requested to undergo an extensive physical examination, including autonomic function tests, on another day. Of this sample, 631 (89%) participated. Subjects were classified according to the WHO criteria, based on the mean values of the two oral glucose tolerance tests [39]: 288 had normal glucose tolerance (NGT), 169 had impaired glucose tolerance (IGT), 95 had newly diagnosed diabetes (NDM), and 79 subjects were known Type 2 diabetic patients (KDM). For evaluating reference values only subjects with a normal glucose tolerance were included. In addition, subjects were excluded because of a self-reported history of neurological disease (5 subjects), self-reported chronic obstructive pulmonary disease (4 subjects), a history of cardiovascular disease (40 subjects) as assessed by means of a Dutch translation of the London School of Hygiene and Tropical Medicine questionnaire [40]. Hypertensive subjects were also excluded; hypertension was defined as current treatment with antihypertensive drugs (49 subjects), mean systolic blood pressure greater than or equal to 160 mmHg, and/or mean diastolic blood pressure greater than or equal to 95 mmHg (36 subjects) based on four blood pressure measurements. This resulted in a study sample of 191 healthy subjects for the computation of the reference values. For the computation of agreement, the data from the complete study sample of 631 subjects were used. Of the 631 initial subjects, 43 were invited to participate in a reproducibility study. The subjects were not selected on health status, but were chosen in such a way to have a wide range of ages and to have both normal glucose tolerance and diabetes. Of the subjects 39 responded, and had a second set of measurements taken within 3 weeks.

Individual data were missing for the following reasons: the test schedule was not completed, the quality of the data was insufficient for processing (a poor blood pressure signal or arrhythmias) or there were more than 10% non-sinus beats in the total number of recorded beats.

The study protocol was approved by the Ethics Committee of the *Vrije Universiteit Medical Centre*. All study participants gave their informed consent.

Participants were asked to refrain from smoking and drinking coffee for 2 h prior to the assessment of cardiovascular autonomic function. Tests took place between 8:30 am and 4:00 pm, at least 1 h after a light meal. Subjects were supine resting in a quiet ambience, with a room temperature between 19 and 22 degrees Celsius. Cardiac cycle duration (RR interval) and continuous finger arterial pressure were measured under three conditions: (i) spontaneous breathing (Fig. 1), (ii) six deep breaths over one minute (Fig. 2), and (iii) an active change in position from lying to standing (Fig. 3). The correct frequency of breathing of six breaths per minute was controlled and dictated by oral and visual instructions of the investigator, who followed the beeps generated by the data-acquisition program. When off-line spectral analysis of the systolic blood pressure data showed a clearly recognizable peak shifted from the breathing frequency of 0.10 Hz by more than 0.02 Hz, the measurements were discarded. After each test a resting period of at least 1 min was included, to prevent influences by previous test conditions.

During the tests, heart rate and blood pressure were continuously recorded on a PC-based data-acquisition system. RR intervals were obtained from a bipolar ECG chest-lead by a hardware QRS detector with an accuracy of one millisecond. Blood pressure was recorded continuously, using the Finapres method (Finger Arterial Blood Pressure, Ohmeda BP2000), digitally sampled at 200 Hz, and off line low-pass filtered and down-sampled to 100 Hz. Beat-to-beat systolic blood pressure values were obtained from this processed blood pressure signal by means of an automatic procedure, which was verified by visual inspection.

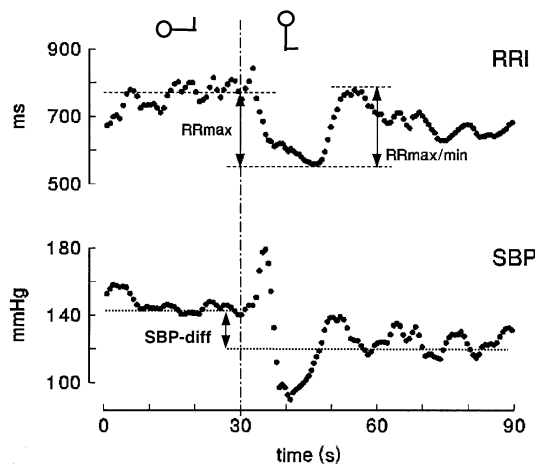


**Fig. 1A, B.** A typical example of beat-to-beat variations in RR intervals (RRI) and systolic blood pressure (SBP) during 3 minutes of spontaneous breathing in the supine position. Subject is a healthy woman aged 57 years. **(A)** Variability in the time domain. **(B)** Variability in the frequency domain, expressed as power spectra; low frequency (LF) and high frequency (HF) band are indicated by horizontal arrows

Ten parameters of cardiac autonomic function were derived from the RR interval and systolic blood pressure recordings obtained during the three respective conditions [37]. During spontaneous breathing over 3 min in the supine position, the mean (mean NN) and the standard deviation (SDNN) of all normal to normal, i.e. sinus rhythm, RR intervals were computed [41]. Further, the power (variance) in the low frequency (LF) band (0.04 to 0.12 Hz) and the power in the high frequency (HF) band (0.12 to 0.40 Hz) were assessed from all normal sinus rhythm RR intervals by spectral analysis [41]. Also, the ratio of the LF power to the sum of the LF and HF power was calculated  $[\text{LF}/(\text{LF}+\text{HF})]$  [41]. From the recording during six deep breaths over 1 min in the supine position, we measured the difference in maximum and minimum RR interval duration during expiration and inspiration, and averaged over the six consecutive breaths (EI difference) [3, 42]. Further, a measure of the sensitivity of the spontaneous baroreflex control of heart rate, baroreflex sensitivity (BRS) was calculated by means of cross-spectral analysis. BRS was defined as the change in RR intervals caused by changes in systolic blood pressure (ms/mmHg), and was estimated as the gain of the transfer function between blood pressure and RR interval changes [43, 44]. For the computation of the BRS only spectral components were used that were within a defined band width (0.05–0.15 Hz) around the

**Fig. 2A–C.** **(A)** Typical example of beat-to-beat variations in RR intervals (RRI) and systolic blood pressure (SBP) during one minute deep breathing at 6 breaths per minute. Same subject as in Figure 1. EI difference is defined as the difference between maximum and minimum RR interval (indicated by the vertical arrow) averaged over 5–6 consecutive breaths; in this case, 245 ms. **(B)** Power spectra of the above recordings. Note that most of the power in RRI and SBP variations is concentrated in the one peak at breathing rate 0.1 Hz (6 per min). **(C)** Transfer function between SBP and RRI variations. The average value of the bold part of the transfer gain function (continuous line) is defined as the baroreflex sensitivity (BRS), in this case, 11.1 ms/mmHg. Note the high coherence (dotted line) in the respiratory band around 0.1 Hz (horizontal arrow)





**Fig. 3.** Typical example of RR intervals (RRI) and systolic blood pressure (SBP) changes during an active change (at  $t=30$  s) in posture from lying to standing. The three autonomic function measures derived from this test are: RRmax, RRmax/min and SBP difference, as is indicated by vertical arrows

breathing frequency of six breaths per minute and with a squared coherence ( $\gamma^2$ ) of 0.5 or higher. During an active change in position from lying to standing, the maximal change in RR interval (RRmax), defined as the difference between the mean RR interval during 1 min prior to standing up and the minimum RR interval within 15 s after standing up, was obtained [17]. Furthermore, we measured the maximum RR interval between 15 and 30 s after standing up divided by the minimum RR interval within 15 s after standing up (RRmax/min) [45] and the systolic blood pressure difference (SBP difference) after standing up calculated as the mean over 30 s during 1.5 to 2 min after standing up, minus the mean over 30 s prior to standing up [3].

**Statistical Analysis.** A normal distribution of SDNN, LF power, HF power, EI difference, BRS, RRmax and RRmax/min values was obtained by taking the natural logarithm. Subsequently, for the presentation of the geometric means, standard deviations and percentiles, ln-values were back-transformed. To assess the possible influence of age and sex, the regression model was used: Autonomic function measure  $=\alpha+\beta_1\cdot\text{age}+\beta_2\cdot\text{gender}+\beta_3\cdot(\text{age}\cdot\text{sex})$  or submodels thereof, depending on the outcome of the analysis; with age in decades and sex as a dichotomous variable, 0=male and 1=female. Logarithmic (ln) transformation of SDNN, LF power, HF power, EI difference, BRS, RRmax and RRmax/min was carried out to obtain normally distributed residuals. Reference values, defined as the 90% prediction interval for individual predictions, were computed from the estimated linear regression parameters, thus optimally using the available measurements [38, 46].

To assess the agreement between the measures of autonomic function, data from all 631 subjects were used. Spearman's correlation coefficient was computed between all ten measures. The computed reference values were applied and subsequently the percentage of findings concordant for autonomic dysfunction was computed.

For the sample, which had duplicate measurements, the reliability coefficient (RC) and the coefficient of variation (CV) were calculated by analysis of variance to obtain test-retest reliability [47].

$\text{RC}=\text{SD}_{\text{between}}^2/(\text{SD}_{\text{between}}^2+\text{SD}_{\text{within}}^2)*100\%$ , with high values of RC representing good reproducibility.  $\text{CV}=\text{SD}_{\text{within}}/$

**Table 1.** Characteristics and measures of autonomic function of the normotensive study sample ( $n=191$ ) stratified by sex in a healthy cohort aged 50 to 75 years<sup>a</sup>

| Characteristics                      | Male ( $n=98$ )            | Female ( $n=93$ )         |
|--------------------------------------|----------------------------|---------------------------|
| Age (years)                          | 62.0 (7.4)                 | 63.3 (7.4)                |
| BMI ( $\text{kg}/\text{m}^2$ )       | 25.4 (2.5)                 | 25.7 (3.5)                |
| Fasting plasma glucose (mmol/l)      | 5.45 (0.51)                | 5.31 (0.49)               |
| HbA <sub>1c</sub> (%)                | 5.35 (0.50)                | 5.31 (0.43)               |
| Cholesterol (mmol/l)                 | 6.47 (1.04)                | 6.67 (1.18)               |
| HDL Cholesterol (mmol/l)             | 1.22 (0.33) <sup>c</sup>   | 1.55 (0.32) <sup>c</sup>  |
| Triglycerides (mmol/l)               | 1.61 (0.77) <sup>c</sup>   | 1.27 (0.60) <sup>c</sup>  |
| Diastolic blood pressure (mmHg)      | 80 (7) <sup>c</sup>        | 77 (7) <sup>c</sup>       |
| Systolic blood pressure (mmHg)       | 128 (12)                   | 128 (13)                  |
| <i>Autonomic function measures</i>   |                            |                           |
| Mean NN, ms                          | 989 (15)                   | 956 (14)                  |
| SDNN, ms <sup>b</sup>                | 36.5 (21.4–63.3)           | 35.4 (18.8–57.9)          |
| LF power, $\text{ms}^2$ <sup>b</sup> | 354 (95–1002) <sup>c</sup> | 213 (51–773) <sup>c</sup> |
| HF power, $\text{ms}^2$ <sup>b</sup> | 189 (44–995)               | 175 (34–964)              |
| LF/(LF+HF), unitless                 | 0.59 (0.02)                | 0.54 (0.02)               |
| EI difference, ms <sup>b</sup>       | 207 (89–385) <sup>c</sup>  | 163 (73–291) <sup>c</sup> |
| BRS, ms/mmHg <sup>b</sup>            | 9.0 (4.5–15.4)             | 8.0 (4.3–14.4)            |
| RR max, ms <sup>b</sup>              | 269 (154–410)              | 246 (162–365)             |
| RR max/min, unitless <sup>b</sup>    | 1.24 (1.08–1.51)           | 1.24 (1.10–1.47)          |
| SBP difference, mmHg                 | –3.8 (1.6)                 | –8.1 (1.6)                |

<sup>a</sup> Data are mean (standard deviation) unless otherwise indicated; <sup>b</sup> Given are the median (10 to 90<sup>th</sup> percentiles); <sup>c</sup> Male vs female,  $p<0.05$  after correction for age

$\text{mean}_{\text{both measurements}}*100\%$ , with low values representing good reproducibility. For the ratio RRmax/min, the CV was computed using  $(\text{mean}_{\text{both measurements}}-1)$  as the nominator.

Two-tailed  $p$  values of less than 0.05 were considered statistically significant.

## Results

**Reference values (in reference sample,  $n=191$ ).** For none of the autonomic function measures the interaction term between age and sex was statistically significant. After adjustment for age, the regression analysis showed that LF power and EI difference were higher for men than for women (Table 1 and 2). Therefore, sex-specific reference values are presented for these measures. Of the ten autonomic function parameters seven decreased with increasing age, while for mean NN, LF/(LF+HF) and SBP difference no association with age was observed. Therefore, we calculated age-specific reference values for SDNN, LF power, HF power, EI difference, BRS, RR max and RR max/min at 50, 55, 60, 65, 70 and 75 years respectively (Table 2).

**Agreement (in whole sample,  $n=631$ ).** The agreement between the ten measures of autonomic function differed widely with correlation coefficients ranging

**Table 2.** Reference values for the ten autonomic function parameters as a function of age<sup>a</sup>. Given are the 90%-prediction intervals for individuals as estimated from a selected healthynormotensive reference sample aged 50 to 75 ( $n=191$ ). Also given are the mean (SD) values for the healthy reference sample (second column)

| Autonomic function measure        | Mean (SD)        |     | Age (years) |         |      |      |      |      |
|-----------------------------------|------------------|-----|-------------|---------|------|------|------|------|
|                                   |                  |     | 50          | 55      | 60   | 65   | 70   | 75   |
| Mean NN, ms                       | 973 (10)         | 5%  | 719         | for all |      |      |      |      |
|                                   |                  | 95% | 1189        | for all |      |      |      |      |
| SDNN, ms <sup>b</sup>             | 36.2 (19.4–60.5) | 5%  | 19.5        | 18.5    | 17.5 | 16.5 | 15.6 | 14.6 |
|                                   |                  | 95% | 82.3        | 77.4    | 72.9 | 68.8 | 65.1 | 61.7 |
| LF power, ms <sup>2b</sup>        | M 354 (95–1002)  | 5%  | 77          | 67      | 58   | 49   | 42   | 36   |
|                                   |                  | 95% | 2595        | 2204    | 1882 | 1614 | 1391 | 1205 |
|                                   | F 213 (51–773)   | 5%  | 56          | 48      | 42   | 36   | 31   | 26   |
|                                   |                  | 95% | 1895        | 1607    | 1369 | 1172 | 1008 | 872  |
| HF power, ms <sup>2b</sup>        | 183 (39–965)     | 5%  | 48          | 38      | 31   | 25   | 20   | 15   |
|                                   |                  | 95% | 2422        | 1913    | 1518 | 1211 | 972  | 784  |
| LF/(LF+HF), unitless              | 0.56 (0.01)      | 5%  | 0.26        | for all |      |      |      |      |
|                                   |                  | 95% | 0.85        | for all |      |      |      |      |
| EI difference, ms <sup>b</sup>    | M 207 (89–385)   | 5%  | 101         | 92      | 83   | 75   | 68   | 61   |
|                                   |                  | 95% | 605         | 544     | 490  | 443  | 401  | 364  |
|                                   | F 163 (73–291)   | 5%  | 84          | 76      | 69   | 62   | 56   | 51   |
|                                   |                  | 95% | 504         | 453     | 408  | 368  | 333  | 302  |
| BRS, ms/mmHg <sup>b</sup>         | 8.8 (4.5–15.0)   | 5%  | 4.7         | 4.3     | 4.0  | 3.6  | 3.3  | 3.0  |
|                                   |                  | 95% | 24.3        | 22.0    | 20.0 | 18.3 | 16.7 | 15.3 |
| RR max, ms <sup>b</sup>           | 256 (158–393)    | 5%  | 161         | 154     | 147  | 140  | 133  | 126  |
|                                   |                  | 95% | 492         | 467     | 445  | 424  | 404  | 386  |
| RR max/min, unitless <sup>b</sup> | 1.24 (1.09–1.49) | 5%  | 1.13        | 1.09    | 1.06 | 1.03 | 1.00 | 0.97 |
|                                   |                  | 95% | 1.63        | 1.58    | 1.53 | 1.48 | 1.44 | 1.40 |
| SBP difference, mmHg              | –5.8 (1.1)       | 5%  | –31         | for all |      |      |      |      |
|                                   |                  | 95% | 22          | for all |      |      |      |      |

<sup>a</sup> For three out of the ten autonomic function parameters no (statistically) significant association with age was observed: mean NN, LF/(LF+HF) and SBP difference. For SDNN, LF power, HF power, EI difference, BRS, RR max and RR

max/min are reported at 50, 55, 60, 65, 70 and 75 years respectively; <sup>b</sup> Ln transformed; given are the median (10<sup>th</sup> to 90<sup>th</sup> centiles)

**Table 3.** Agreement between the measures of autonomic function in the complete sample of  $n=631$ . The upper right part shows the Spearman correlation coefficients. The lower leftpart gives the percentage of concordant grouping when applying the reference values (lowest 5<sup>th</sup> centile)

| Autonomic function measures | Mean | SDNN              | LF power          | HF Power          | LF/(LF+HF)         | EI difference     | BRS               | RR max             | RR max/min        | SBP difference    |
|-----------------------------|------|-------------------|-------------------|-------------------|--------------------|-------------------|-------------------|--------------------|-------------------|-------------------|
| Mean NN                     |      | 0.45 <sup>a</sup> | 0.32 <sup>a</sup> | 0.45 <sup>a</sup> | –0.19 <sup>a</sup> | 0.21 <sup>a</sup> | 0.38 <sup>a</sup> | 0.71 <sup>a</sup>  | 0.29 <sup>a</sup> | 0.09 <sup>b</sup> |
| SDNN                        | 42%  |                   | 0.82 <sup>a</sup> | 0.80 <sup>a</sup> | –0.07              | 0.52 <sup>a</sup> | 0.48 <sup>a</sup> | 0.52 <sup>a</sup>  | 0.41 <sup>a</sup> | 0.16 <sup>a</sup> |
| LF power                    | 26%  | 56%               |                   | 0.70 <sup>a</sup> | 0.26 <sup>a</sup>  | 0.51 <sup>a</sup> | 0.43 <sup>a</sup> | 0.45 <sup>a</sup>  | 0.39 <sup>a</sup> | 0.10 <sup>b</sup> |
| HF power                    | 29%  | 56%               | 52%               |                   | –0.46 <sup>a</sup> | 0.49 <sup>a</sup> | 0.43 <sup>a</sup> | 0.48 <sup>a</sup>  | 0.40 <sup>a</sup> | 0.13 <sup>a</sup> |
| LF/(LF+HF)                  | 11%  | 6%                | 18%               | 2%                |                    | –0.01             | –0.02             | –0.11 <sup>b</sup> | –0.06             | –0.04             |
| EI difference               | 27%  | 50%               | 48%               | 37%               | 15%                |                   | 0.69 <sup>a</sup> | 0.35 <sup>a</sup>  | 0.33 <sup>a</sup> | 0.08              |
| BRS                         | 48%  | 48%               | 38%               | 36%               | 14%                | 63%               |                   | 0.37 <sup>a</sup>  | 0.31 <sup>a</sup> | 0.06              |
| RR max                      | 87%  | 55%               | 41%               | 41%               | 7%                 | 31%               | 31%               |                    | 0.57 <sup>a</sup> | 0.16 <sup>a</sup> |
| RR max/min                  | 13%  | 15%               | 16%               | 13%               | 2%                 | 17%               | 13%               | 18%                |                   | 0.05              |
| SBP difference              | 0%   | 11%               | 11%               | 3%                | 7%                 | 8%                | 8%                | 3%                 | 3%                |                   |

<sup>a</sup>  $p < 0.01$ ; <sup>b</sup>  $p < 0.05$

from –0.01 to 0.82 and, as assessed by applying the reference values (Table 2) to the total sample ( $n=631$ ), the percentage of findings concordant for autonomic dysfunction ranged from 0% to 87% (Table 3). The agreement and correlation coefficient of

LF/(LF+HF) and SBP difference with all other autonomic function measures were the lowest. RRmax/min also showed a markedly low percentage of concordance with the other measures, while Spearman's correlation coefficients were moderate. For

**Table 4.** Reproducibility of ten measures of autonomic function in a sample of  $n=36$ , taken out of the complete sample of  $n=631$  of 50- to 75-year-old subjects, expressed as the SD

within subjects, SD between subjects, reliability coefficient (RC), and coefficient of variation (CV)

| Autonomic function measure | N  | Mean <sup>a</sup> | SD within | SD between | RC (%) | CV (%) |
|----------------------------|----|-------------------|-----------|------------|--------|--------|
| Mean NN, ms                | 36 | 911 (185)         | 72        | 172        | 85     | 8      |
| SDNN, ms                   | 36 | 30.0 (12.9)       | 8.9       | 9.4        | 53     | 30     |
| LF power, ms <sup>2</sup>  | 36 | 288 (298)         | 218       | 205        | 47     | 76     |
| HF power, ms <sup>2</sup>  | 36 | 189 (201)         | 133       | 152        | 56     | 71     |
| LF/(LF+HF), unitless       | 36 | 0.59 (0.17)       | 0.13      | 0.12       | 47     | 22     |
| EI difference, ms          | 38 | 181 (101)         | 73        | 70         | 47     | 40     |
| BRS, ms/mmHg               | 36 | 7.7 (3.4)         | 2.6       | 2.3        | 43     | 34     |
| RR max, ms                 | 36 | 209 (117)         | 30        | 114        | 93     | 14     |
| RR max/min, unitless       | 36 | 1.22 (0.16)       | 0.09      | 0.13       | 71     | 39     |
| SBP difference, mmHg       | 36 | -9.76 (14.04)     | 10.71     | 11.82      | 55     | 110    |

SDNN, LF power, HF power, EI difference, BRS and RRmax agreement was good.

*Reproducibility (in reproducibility sample,  $n=39$ ).* In a subset of the Hoorn Study sample ( $n=36$  to 38) from which duplicate measurements were available, the mean values, SD within subjects and SD between subjects, reliability coefficient (RC) and coefficient of variation (CV) were calculated for all autonomic function measures (Table 4). In general, reproducibility of all parameters was moderate to high, with RCs ranging from 43 to 93% and CVs ranging from 7 to 110%. Mean NN and RRmax had the best reproducibility with a RC over 85% and CV below 10%.

## Discussion

This study shows data on age-specific and sex-specific reference values as well as reproducibility and mutual agreement of ten measures of autonomic function in adults aged 50 to 75 years. With the exception of two, the agreement between the measures of autonomic function was fairly good.

*Reference values.* This study, which is based on a screening with repeated oral glucose tolerance tests in a random sample from a well-defined general population. It has, therefore, the major advantage of not including subjects with impaired autonomic function due to impaired glucose tolerance or undiagnosed diabetes mellitus. We excluded subjects with other characteristics known to be associated with impaired autonomic function, i.e. a history of cardiovascular disease, hypertension and use of certain medication, or neurological disease. Moreover, our sample ( $n=191$ ) includes a substantial number of subjects in a high age category, which has been sparsely shown in previous studies, despite its high relevance in view of the common occurrence of Type 2 diabetes and its complications in this age group. Furthermore, this is the first large study in which three different categories

of autonomic function measures were studied simultaneously: Ewing-test measures, heart-rate variability measures and baroreflex sensitivity.

The association between measures of autonomic function and sex is consistent with previous findings: women had lower values of autonomic function [9, 14, 15]. This association was both statistically and clinically significant for LF power and EI difference and appropriate sex-specific reference values were given. Also, the association between measures of autonomic function and age is consistent with previous findings [8, 9, 10, 11, 12, 13], although we found slightly lower associations with age. This could be attributable to the older age of our subjects compared to previous studies. Our study extends previous findings in a sample of more advanced age.

It has been reported that high values of autonomic function measures also have a predictive value in cardiovascular disease; high values are associated with a higher risk of cardiovascular disease [48]. Therefore, we have provided both the lower and upper reference values. We have computed the reference values on a statistical basis, by taking the upper and lower five percent of individual predictions on the basis of the estimated variances. Ideally, one would define a cut-off value on the basis of subjects who get the disease and those who do not get the disease. In case of autonomic neuropathy however, this is difficult since there is no consensus about the diagnosis of the disease. Nevertheless, several papers call for standardization of measurements [1, 2]. Furthermore, the two distribution curves of the groups with and without the disease are not likely to be completely separated, and some statistical decision will have to be made eventually. Therefore, the chosen statistical approach is the most feasible with the current knowledge and consensus.

Of course it would be of utmost importance to use the defined criteria to assess the prevalence and incidence of autonomic dysfunction in the general population. However, because the Hoorn Study is an age-, sex- and glucose-tolerance-stratified cohort we were not able to do this. Several other studies [26] would

provide excellent cohorts to apply these reference values. The predictive value for mortality and morbidity, however, would be more informative, as well as longitudinal observations on changes in autonomic function. *Agreement.* The agreement, as assessed in the non-selected sample of 631 subjects, varied greatly between the different measures of autonomic function. One prominent finding was the high agreement between SDNN, LF power, BRS and EI difference with the other measures of autonomic function.

The measures derived from the lying-to-standing test, however, did poorly agree with the measures of the other two tests. It could be that the lying-to-standing test depends, more than the other tests, on the patient's co-operation or ability to exert effort. Especially in an elderly population this could be problematic, as is also shown by the higher number of missing values for this test. Poor agreement between heart-rate changes during deep breathing and after standing up has also been reported in a study of 133 healthy subjects in the age range of 10 to 65 years, where they found a correlation between EI difference and RR max of 0.17 and between EI difference and RR max/min of 0.14 [17]. An explanation might be the possible involvement of different afferent and efferent mechanisms [49]. For instance the RRmax/min measure expresses the ratio of the relative bradycardia and the immediate occurring tachycardia after standing up. While the latter is almost exclusively determined by parasympathetic withdrawal, the bradycardia is a rebound effect depending on the presence of a recovery (and overshoot) of blood pressure by sympathetically mediated vasoconstriction [50]. This makes the RRmax/min ratio an autonomic function parameter expressing cardiac vagal as well as (indirect) vasomotor sympathetic functioning, while deep breathing heart rate changes are almost exclusively vagally mediated. Similar arguments as for the RRmax/min, hold for the systolic blood pressure fall after standing up (SBP difference). Moreover, SBP difference probably measures more than just autonomic function, as the compliance of the vascular (venous) system could play a role as well.

Another remarkable observation was the lack of agreement between the spectral HRV power ratio, the LF/(LF+HF) measure, and all other measures of autonomic function. In 1986, it was suggested that the instantaneous balance between sympathetic and vagal nerve activities might be captured by a single number [51]. This ratio, or so-called sympathovagal balance, has been embraced by many others, because it would offer "new possibilities for understanding dynamic, critically important autonomic interrelations in humans by use of totally non-invasive, unobtrusive means" [52]. Although applied by many investigators in the field of autonomic neural functioning, the concept of this balance with its lack of a sound physiological basis, also has been seriously criticized [53, 54]. Our results cast serious doubts on this power ratio as a measure of autonomic function,

since it shows little to no agreement with any of the other measures of autonomic function.

Furthermore, we observed that measures obtained from within the same test showed a higher agreement than with those from another test. This is likely due to the fact that these measures are obtained from the same data set. For instance, the SDNN measure is mathematically closely related to the spectral power (the total power equals variance, which is by definition the squared standard deviation); so it is to expect that the SDNN measure taken from the spontaneous three minutes of HRV agrees relatively well with the LF and HF power computed from this same three minutes of HRV data, which indeed it does, given the correlation coefficients of 0.82 and 0.80, respectively.

We applied two different measures to express the agreement between the autonomic function measures: Spearman's non-parametric correlation coefficients and the percentage of agreement in subjects labelled as having autonomic dysfunction based on the reference values. The latter gives additional information, since correlation coefficient alone strictly speaking gives only the strength of a relation between two variables and not the agreement. [55] Both the correlation coefficients and the percentages of agreement gave very similar results, indicating that the agreement was similar not only in the lower tail of the distribution, but over the entire range.

*Reproducibility.* In general, reproducibility, as assessed in the subsample of 39 subjects, was moderate to high. For example, the EI difference, the most frequently used measure for autonomic function, had a RC of 47% and a CV of 40%. BRS and HRV measures (SDNN, LF power, HF power) showed a comparable reproducibility. These observations are roughly in line with other studies. Best reproducibility was observed for the Mean NN and the RR max with RCs over 85% and CVs below 10%. The observed moderate reproducibility could be partly attributable to the advanced age of the subjects in two ways. Firstly, because lower mean values are inherent to higher age which directly leads to higher CVs (see methods section for formula). Secondly, elderly people in general have more difficulties to correctly carry out certain tests (deep breathing and standing up quickly enough). This becomes apparent when comparing our reproducibility with other studies: somewhat higher reproducibility for the deep breathing test (EI difference and BRS) for slightly younger subjects [12, 56, 57, 58]. Reproducibility of course might be improved by standardizing the conditions during measurements [56]. In our study measurements took place between 8:30 am and 4:00 pm. Since there was no further standardization regarding time it is possible that diurnal rhythm has influenced the reproducibility to some extent. However it will not have caused a systematic difference, since there was no systematic approach in the sequence of measurements with respect to for example age or glucose tolerance.



Our reproducibility data are probably more indicative of autonomic function testing in daily clinical practice compared to "clean" laboratory studies and in that sense might be more valuable.

Some earlier attempts have been made to compare classic Ewing tests with some of the new HRV or BRS in studies of autonomic dysfunction in mainly Type 1 diabetes [4, 16, 20, 59]. This study is, however, the first that compares standard Ewing tests, HRV as well as BRS within the same sample at an advanced age and not restricted to diabetic patients. The correlation coefficients and percentage concordance showed that the agreement between the tests was not perfect. This might on one hand be due to the moderate reproducibility and on the other hand different measures could (partly) measure different physiological substrates. In the latter case some measures may be complementary to each other. In view of these results it would be recommended to use a combination of measures to assess autonomic dysfunction.

In conclusion, this study shows that the agreement between eight out of ten measures of cardiovascular autonomic function varied greatly. Application of reference values of autonomic function may be considered for testing autonomic function: age- and sex specific reference values are given for adults aged 50 to 75 years.

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